BRIEF REPORT

Clinical Care of Two Patients with Ebola Virus Disease in the United States

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SUMMARY

West Africa is currently experiencing the largest outbreak of Ebola virus disease (EVD) in history. Two patients with EVD were transferred from Liberia to our hospital in the United States for ongoing care. Malaria had also been diagnosed in one patient, who was treated for it early in the course of EVD. The two patients had substantial intravascular volume depletion and marked electrolyte abnormalities. We undertook aggressive supportive measures of hydration (typically, 3 to 5 liters of intravenous fluids per day early in the course of care) and electrolyte correction. As the patients' condition improved clinically, there was a concomitant decline in the amount of virus detected in plasma.

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THE LARGEST OUTBREAK OF EVD IN HISTORY BEGAN IN DECEMBER 2013 IN Guinea, a country in West Africa.[1] By late March, Liberia had reported seven cases. By the end of May, the epidemic had spread to Sierra Leone. As of November 5, 2014, a total of 13,042 cases of EVD (including 4818 deaths) had been reported in six countries in West Africa (Guinea, Sierra Leone, Liberia, Mali, Nigeria, and Senegal), the United States, and Spain.[2]

EVD causes a nonspecific febrile illness associated with myalgia, with progression to gastrointestinal symptoms (abdominal pain, nausea, vomiting, and diarrhea). In the second week of illness, hemorrhagic symptoms and sepsis may develop.[3-5] Mortality from EVD historically has ranged from 40 to 88%.[3,4,6-8] The current outbreak is attributed to *Zaire ebolavirus* (EBOV). The aggregated case fatality rate associated with EBOV is 78%, which is higher than that for other Ebola virus species.[4,8,9] Here, we review the clinical course of two American health care workers who contracted EVD in Liberia and were transferred to Emory University Hospital for continued management.

CASE REPORTS

PATIENT 1

The first patient was a 33-year-old physician who had been working in Liberia since October 2013, during which time he had remained healthy while taking daily combination therapy with atovaquone and proguanil as prophylaxis against malaria. In April 2014, he and his team established an EVD care unit in Monrovia, and patients with confirmed EVD began arriving at this facility on June 11, 2014. On July 23, 2014, he awoke feeling febrile and fatigued; his oral temperature was 37.8°C. He

reported his symptoms to colleagues and remained at home. Results on two rapid diagnostic tests for malaria (Standard Diagnostics) were negative. He started empirical malaria treatment with artemether and lumefantrine. Later that day, his oral temperature was 38.6°C, and nausea developed. He was tested for malaria by means of a rapid diagnostic test and for yellow fever, Lassa fever, and EBOV by means of semiquantitative real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assays, all of which were performed at the Liberian National Reference Laboratory. The results for all the tests were negative.

As his fevers continued, intravenous lactated Ringer's solution and empirical antibiotics were administered. On day 4 of the illness, repeat blood testing for malaria, yellow fever, Lassa fever, and EBOV showed positive results for EBOV. On day 6, a petechial rash developed on his arms and chest, his fever spiked at 40.3°C, and he had increasing malaise. Abdominal pain and profuse diarrhea also developed. The rash progressed to a maculopapular rash covering his body from legs to face. He also had an episode of melena and received 1 unit of whole blood. On day 7, he had hematemesis and received another unit of whole blood. Later the same day, he received 1 unit of convalescent whole blood from a patient who had recovered from EBOV. However, his condition continued to worsen. For fever and myalgia, he received 1 g of acetaminophen every 6 hours. He hydrated orally with Tang and Gatorade, despite persistent anorexia. On day 9, he received an intravenous dose of ZMapp, an experimental cocktail of three EBOV glycoprotein-specific monoclonal antibodies (Mapp Biopharmaceutical and LeafBio). The medical team caring for him reported improvements in his vital signs and alertness within 8 hours after the infusion of the monoclonal antibody cocktail. In addition, the extent of the rash decreased, and the patient reported that his energy level had increased to the point that he was able to walk.

On day 10, he was transferred to the Serious Communicable Diseases Unit (SCDU) at Emory University Hospital in Atlanta. On arrival at the hospital on day 11, he was febrile (temperature, 38.9°C) and had tachycardia (≥120 beats per minute), but the blood pressure was stable, with mean arterial pressures of 67 to 103 mm Hg. He required oxygen supplementation at a rate of 2 liters per minute during the first 48 hours, since his oxygen saturation by pulse oximetry (SpO_2) was 91 to 93% while he was breathing ambient air. There was clinical evidence of hypovolemia (orthostasis and resting tachycardia) despite 3+ pitting edema to the waist. He had no signs of bleeding but had a persistent petechial rash. He had decreased breath sounds and dullness on percussion in the right lower thorax, findings that were consistent with a small pleural effusion, although no chest radiography was performed to confirm this finding. There was mild tenderness on palpation in the right upper quadrant of the abdomen, but the liver span was normal. Laboratory examinations were performed in the SCDU with the use of point-of-care instrumentation. Figure 1 shows the changes in pertinent laboratory values over the duration of the hospital stay.

A point-of-care measurement showed an international normalized ratio of 1.3. The patient's kidney function was normal throughout hospitalization. A plasma specimen that was collected on admission to the hospital tested positive for EBOV on semiquantitative RT-PCR assay at the Centers for Disease Control and Prevention (CDC). The lowest platelet count (51,000 per cubic millimeter) occurred on day 14 of the illness. Immediately after admission, the patient underwent intravenous volume resuscitation, which was initially performed with normal saline, with fluids changed later to 5% dextrose and half-normal saline with potassium chloride.

After initial fluid resuscitation, frequent premature ventricular contractions at up to 6 episodes per minute developed. The patient was found to have hypokalemia (potassium level, 3.0 mmol per liter; lower limit of the normal range, 3.6 mmol per liter). He received 80 to 100 mmol of oral or intravenous potassium chloride per day for the first 5 days of hospitalization. He continued to have diarrhea, with a total output of 2 to 4 liters per day. The goals of his care were to balance fluid input and output and to correct electrolyte disturbances. In order to replace fluid losses from diarrhea, he required 2 to 5 liters of intravenous fluids per day (plus oral intake) until day 17. In addition, his dietary intake was supplemented with oral protein drinks and a multivitamin.

The patient received additional infusions of the antibody cocktail on days 12 and 15 of the illness, without adverse effects. On day 15 of the illness, he became afebrile. Between days 14 and 17,

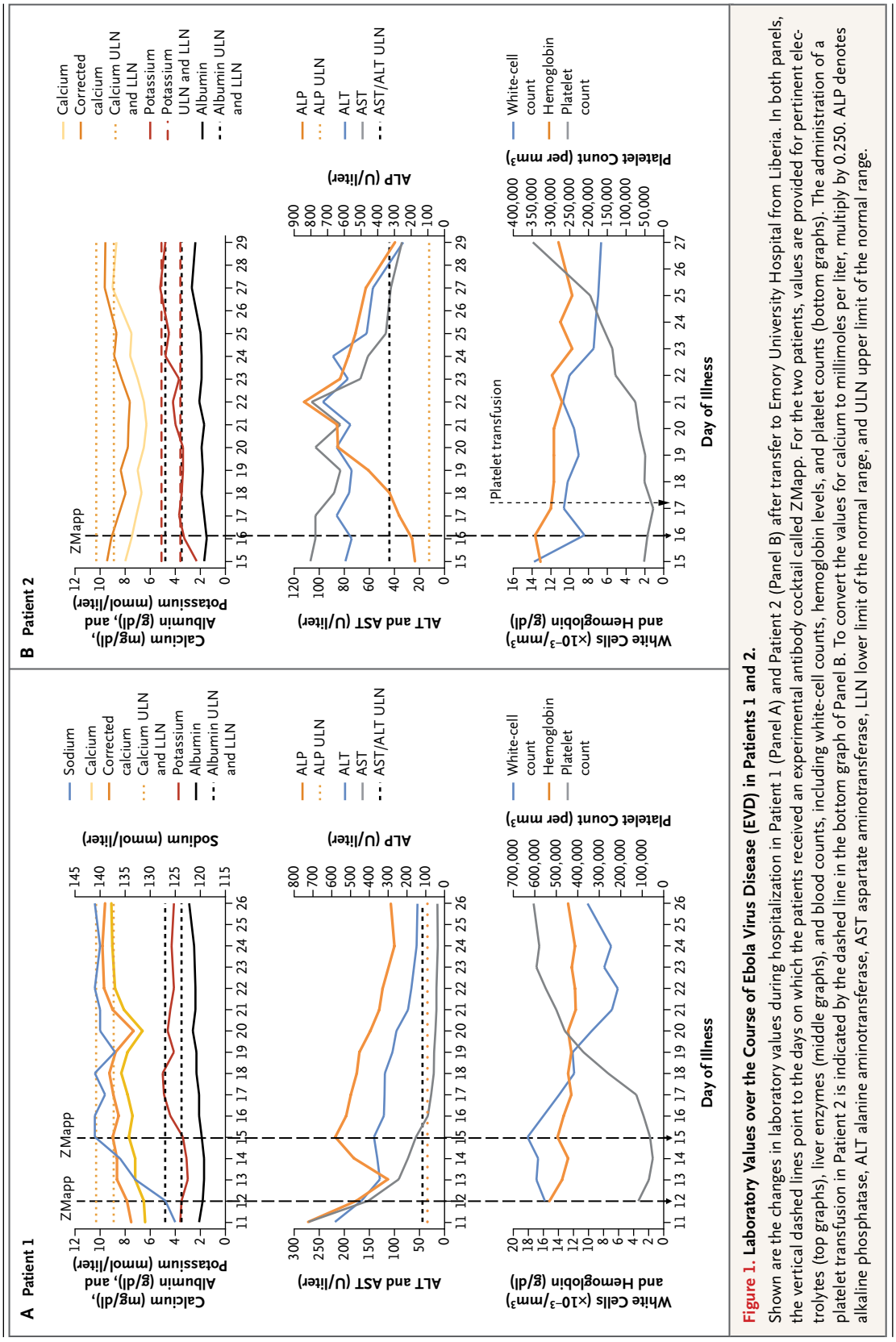

Figure 1. Laboratory Values over the Course of Ebola Virus Disease (EVD) in Patients 1 and 2.

Shown are the changes in laboratory values during hospitalization in Patient 1 (Panel A) and Patient 2 (Panel B) after transfer to Emory University Hospital from Liberia. In both panels, the vertical dashed lines point to the days on which the patients received an experimental antibody cocktail called ZMapp. For the two patients, values are provided for pertinent electrolytes (top graphs), liver enzymes (middle graphs), and blood counts, including white-cell counts, hemoglobin levels, and platelet counts (bottom graphs). The administration of a platelet transfusion in Patient 2 is indicated by the dashed line in the bottom graph of Panel B. To convert the values for calcium to millimoles per liter, multiply by 0.250. ALP denotes alkaline phosphatase, ALT alanine aminotransferase, AST aspartate aminotransferase, LLN lower limit of the normal range, and ULN upper limit of the normal range.

the frequency and volume of stools decreased; stools were formed on day 17, and intravenous fluids were stopped. On day 29, he was removed from isolation after two consecutive plasma specimens that were collected at least 24 hours apart tested negative for EBOV on semiquantitative RT-PCR assay. He was discharged the following day.

PATIENT 2

The second patient was a 59-year-old female missionary who had been working in Liberia at the same facility as Patient 1. She assisted health care workers with donning and doffing personal protective equipment and performing decontamination. On July 22, 2014, she noted the onset of fever, fatigue, and malaise. A blood smear for malaria was positive, and she was prescribed artemether–lumefantrine for 4 days. However, she continued to be febrile, with a peak temperature of 39.4°C. Empirical ceftriaxone was started, but a truncal rash developed. Oral azithromycin was substituted but caused hand swelling; her treatment was successfully changed to oral levofloxacin. A serum sample that was obtained on day 5 of the illness yielded a positive result for EBOV on RT-PCR assay. On day 9, asthenia that required assistance with walking developed, as well as diarrhea without nausea or vomiting. Despite anorexia, the patient continued to drink fluids, including oral rehydration solution. On day 10, she received an infusion of the experimental ZMapp antibody cocktail. She had some subjective improvement in energy within 8 hours after the infusion, and her appetite improved on day 11 such that she could eat solid food, but she remained fatigued and weak. On day 12, she was noted to have some minor bleeding from her nose and venipuncture sites; the hemoglobin level was 11.5 g per deciliter, and she received 1 unit of whole blood. She received a second infusion of the antibody cocktail without adverse effects on day 13.

On day 14, the patient was medically evacuated from Liberia to Emory University Hospital and arrived on day 15. During transport, intravenous access could not be obtained owing to anasarca. On admission to the SCDU, she had evidence of hypovolemia (initial central venous pressure, 3 cm of water, after the placement of a central venous catheter in the right internal jugular vein) despite 3+ pitting anasarca. She was afebrile and remained without fever throughout hospitalization. She had intermittent tachycardia, at up to 104 beats per minute, but with mean arterial pressures (on cuff readings) that were maintained at 87 to 119 mm Hg. She had intermittent tachypnea, with up to 25 breaths per minute, although the SpO_2 was maintained at more than 92%, with oxygen supplementation of 1 to 3 liters per minute by nasal cannula. The laboratory examinations showed both hypokalemia and hypoalbuminemia. (All laboratory data are provided in Fig. 1.)

The patient's kidney function remained normal throughout her hospitalization. A rapid malaria test (BinaxNOW Malaria, Alere) was negative. A plasma specimen that was collected on day 15 tested positive for EBOV on semiquantitative RT-PCR assay at the CDC. The patient received intravenous rehydration with normal saline, which was subsequently changed to 5% dextrose and half-normal saline, with 40 mmol of potassium chloride and oral potassium supplementation. Over the course of the first 2 days at our hospital, she required 2 to 3 liters of intravenous fluids in addition to oral fluids and 60 to 80 mmol of potassium chloride per day. The steady increase in alkaline phosphatase levels was attributed to hypocalcemia (Fig. 1). However, we could not fractionate the alkaline phosphatase to confirm bone as the source. The patient had thrombocytopenia on arrival but did not require the transfusion of 1 unit of platelets (O negative) until day 17. On day 16, the patient received a third dose of the experimental ZMapp antibody cocktail. She had a few loose stools between days 12 and 20 but no diarrhea. Peripheral neuropathy developed in both feet, which responded to gabapentin. After 20 days of illness, her anorexia and asthenia improved. She was discharged from the hospital on day 29, after two consecutive plasma specimens that were collected at least 24 hours apart were negative for EBOV on semiquantitative RT-PCR assay. At the time of discharge, she was able to walk without assistance.

CLINICAL FOLLOW-UP

The two patients received ZMapp under emergency investigational new drug approvals from the Food and Drug Administration; both provided written informed consent, originally in Liberia and again on arrival in the United States. The

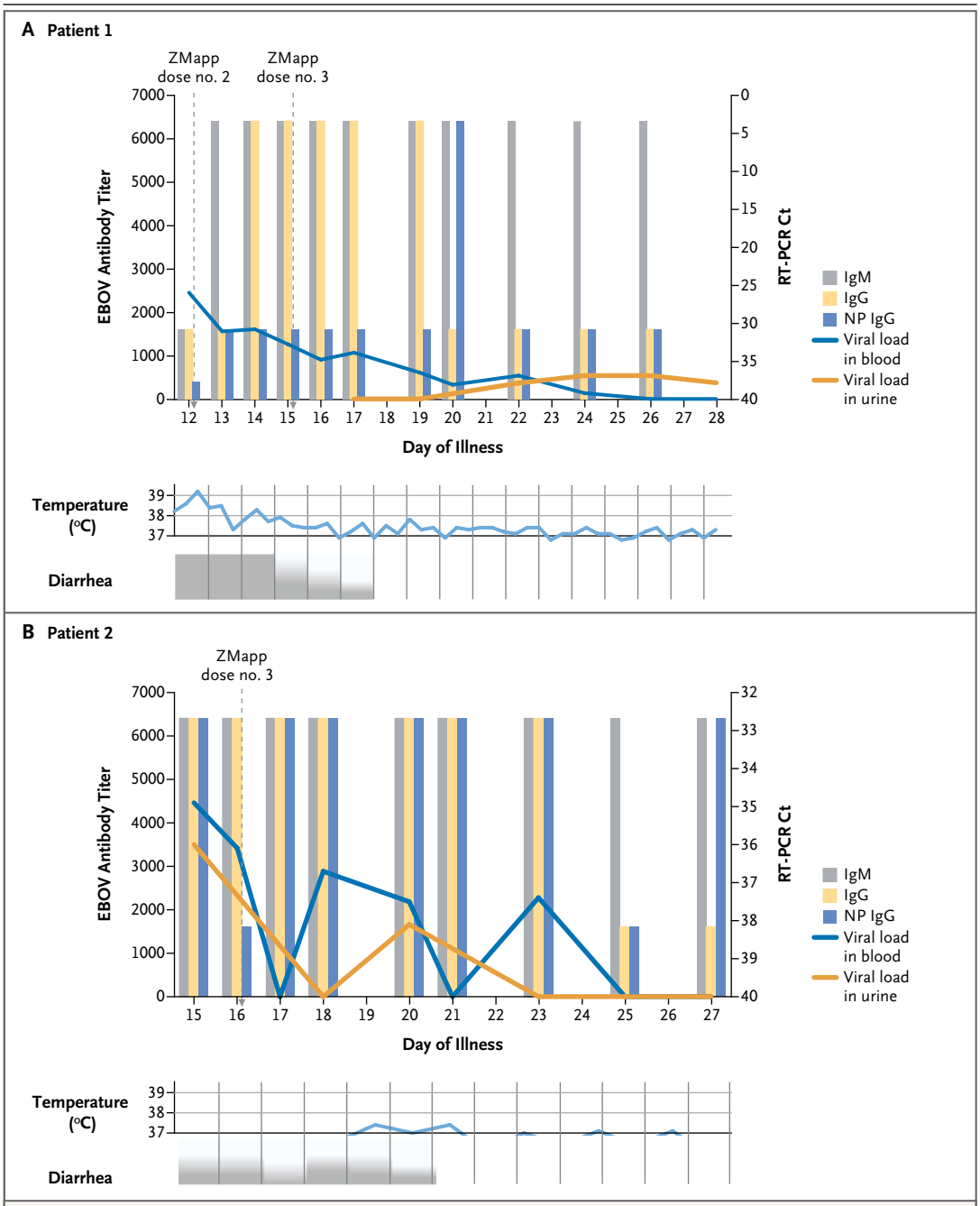

Figure 2. Association between EBOV-Specific Antibodies, Viral Load, and EVD Symptoms.

Shown is the association between the development of specific antibodies against *Zaire ebolavirus* (EBOV), the reduction in the viral load in blood and urine, and EVD-related symptoms in Patient 1 (Panel A) and Patient 2 (Panel B). The reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay targets the nucleoprotein (NP) sequences of EBOV, and the results for viral load are shown as cycle threshold (Ct) values (i.e., the number of cycles at which the fluorescence in the assay exceeds the threshold). The Ct value is inversely related to the viral load. IgM and IgG titers on enzyme-linked immunosorbent assay (ELISA) against an EBOV lysate are shown. However, the titers may include antibodies from both the monoclonal antibody cocktail (ZMapp) that was administered and endogenous EBOV-specific antibodies. To evaluate the kinetics of the host-specific antibodies, an ELISA was developed to detect antibodies against the EBOV NP antigen, which would not be contained in the monoclonal antibody cocktail. These data indicate that the production of EBOV-specific antibodies is associated with a reduction in the viral load in the blood and urine, and the reduction in the viral load is associated with the resolution of EVD-related symptoms.

two patients had a decline in the plasma EBOV viral load (with a corresponding increase in cycle threshold values on semiquantitative RT-PCR assay) during hospitalization. EBOV was isolated from a blood specimen that was collected from Patient 1 on the first day of hospitalization (illness day 11). EBOV nucleic acid was detectable in plasma and urine for nearly 4 weeks after the onset of illness. The two patients had detectable IgM and IgG antibodies after the second week of illness. Figure 2 shows the results of semiquantitative RT-PCR assays for EBOV and immunoglobulin responses in the two patients. In general, there was a correlation between increasing antibody levels and decreasing cycle threshold values on RT-PCR assay.

Patient 1 and Patient 2 each had a follow-up evaluation 4 weeks after discharge. Both patients reported a continued increase in strength and stamina. Patient 2 reported resolution of peripheral neuropathy, and she was given instructions on tapering gabapentin.

DISCUSSION

Two patients with EVD who were treated at Emory University Hospital had hypovolemia, hypokalemia, hypocalcemia, and hypoalbuminemia. Patient 1 also had hyponatremia. Both patients had thrombocytopenia without evidence of coagulopathy. With aggressive fluid and electrolyte replacement, the condition of both patients improved. The clinical benefit of the experimental monoclonal antibody therapy in these patients is unknown. The two patients were observed to have subjective and objective improvement shortly after receiving the first dose of the antibody cocktail, but this improvement occurred in the context of receiving other care as well. Studies in animals have shown a survival benefit for ZMapp even when treatment was initiated after the onset of symptoms.[10] However, there are currently no data on the safety or efficacy of ZMapp in humans. Clinical improvement in these patients could have resulted from a direct effect of the antibodies, from improvement in fluid status through increased oncotic pressure, or from other unidentified factors. Controlled clinical trials are needed to assess the efficacy of ZMapp for EVD.

Although it is likely that most deaths from EVD are caused by multiorgan dysfunction and septic shock or disseminated intravascular coagulation and bleeding complications,[7,11,12] we hypothesize that a subgroup of patients may die from complications of hypovolemia and concomitant electrolyte derangement, primarily hypokalemia. Patient 1 had some ventricular ectopy, so we suspect that electrolyte abnormalities and volume shifts could cause cardiac arrhythmias and sudden death from cardiac causes in some patients.

Our experience with these two patients builds on published data involving patients with EVD. Rollin et al.[13] reported that the development of liver failure (as shown by an elevated level of aspartate aminotransferase but not of alanine aminotransferase) or renal failure was associated with an increased risk of death.[13] One of our patients had substantial liver injury but with good hepatic synthetic function and good kidney function maintained. Rollin et al. reported that hypoalbuminemia, hypocalcemia, and elevated amylase D-dimer levels were all associated with increased mortality among patients with EVD caused by *Sudan ebolavirus* (SUDV).[13] However, the two patients in our study had hypoalbuminemia and elevated alkaline phosphatase levels, which should be predictive of an increased risk of death.[13] In addition, their serum calcium levels were near the levels that were found by Rollin et al. to be associated with increased mortality.[13]

In our patients, aggressive volume and electrolyte replacement with a special focus on replacing potassium and calcium appeared to be of value. Although we believe that aggressive hydration is important, the vascular leak syndrome can lead to substantial pooling of fluid in the third space, including pleural effusions. This finding suggests the need for adjustment of fluid replacement on the basis of the patient's respiratory status. Although the oral rehydration solution recommended by the World Health Organization contains some potassium,[14] most intravenous fluids that are recommended for rehydration do not have substantial levels of potassium, calcium, or magnesium.[14] Rehydration with commercial sports drinks may increase the risk of hypokalemia.[15] Thus, our findings suggest that it may be prudent to supplement oral rehydration with oral potassium, calcium, and magnesium, especially in patients with large-volume diarrhea.

Measurement of viral loads and testing for antibody responses to EBOV were performed only after the two patients arrived in the United States, and both patients had evidence of EBOV-

specific IgM and IgG antibody levels at that time (Fig. 2). In a previous study, EBOV IgM and IgG antibodies were detectable at 8 to 10 days after the onset of illness, with the IgM antibody peak at 18 days.[16] SUDV IgG is detectable for up to 12 years.[16,17] In both of our patients, plasma EBOV loads decreased over time, in correlation with the resolution of clinical illness and laboratory abnormalities. Nevertheless, EBOV RNA continued to be detectable until the fourth week of illness. It is unclear whether this represents persistence of infectious virus or simply detectable RNA.

Both of our patients received whole-blood transfusions in Liberia, including (in the case of Patient 1) blood from an EVD survivor. Although convalescent serum from Ebola survivors has been administered previously, no inferences with respect to its benefits are possible without a controlled clinical trial.[18,19] Patients with EVD and coagulopathy benefit from infusion of fresh-frozen plasma and platelets.[20,21] Some experts recommend the early use of platelet transfusions, since platelets produce the majority of the soluble CD40 ligand that is present in the blood.[21] Such use has been correlated with survival in SUDV-infected patients.[21] Therefore, the ability to fractionate and administer blood products safely may be beneficial to patients in this epidemic.

Our limited experience with two patients cannot be extrapolated to all patients with EVD. However, intensive care nursing, aggressive oral and intravenous rehydration, electrolyte supplementation, and transfusion of blood products appeared to be critical for a positive outcome in our patients with EVD.

The views expressed in this article are those of the authors and do not necessarily represent the official position of the CDC.

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